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PREVALENCE OF ANTIBODIES AGAINST *TOXOPLASMA GONDII* IN POLAR BEARS (*URSUS MARITIMUS*) FROM SVALBARD AND EAST GREENLAND

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ABSTRACT: Serum samples from 419 polar bears (*Ursus maritimus*) from Svalbard and the Barents Sea (collected 1990–2000) and 108 polar bears from East Greenland (collected 1999–2004) were assayed for antibodies against *Toxoplasma gondii* using the modified agglutination test. Antibody prevalences were 3.6% among cubs dependent on their mothers and 21.4% among subadults and adults. Among subadults and adults there was an interaction between population and sex, with similar prevalences among females (Svalbard = 19.5%, Greenland = 18.0%), but a high frequency among Svalbard males (28.7%) as compared to Greenland males (5.8%). The pattern was also significant after correcting for differences in age distribution. The sex-population interaction term is believed to be connected to area- and sex-specific feeding ecology. The prevalences of antibodies against *T. gondii* in Svalbard and Greenland were high compared to previously reported findings in polar bears from Russian and Alaskan areas.

Toxoplasmosis caused by the protozoan parasite *Toxoplasma gondii* is considered a ubiquitous infection affecting all warm-blooded animal species, including humans. Sexual reproduction is known to take place only in the domestic cat and some wild felids. Those animals are regarded as pivotal in the epidemiology of *T. gondii* (Dubey and Beattie, 1988). Remote islands with no cats, such as some Pacific atolls, have been considered free from infection (Wallace, 1973). In agreement with this view, the seroprevalence of *T. gondii* among stray pigs was very low on an island with few cats (Dubey et al., 1997). Asexual reproduction of *T. gondii* takes place in all intermediate hosts; all birds and mammals may, therefore, serve as a source of infection when eaten by other animals (Dubey and Beattie, 1988).

Among marine mammals, antibodies against *T. gondii* have been reported in a range of species from the Atlantic coast of Canada, such as gray seal (*Halichoerus grypus*), harbor seal (*Phoca vitulina*), and hooded seal (*Cystophora cristata*) (Measures et al., 2004), as well as from the Atlantic and Pacific coasts of the United States, e.g., sea otter (*Enhydra lutris*), Pacific harbor seal (*Phoca vitulina richardsi*), sea lion (*Zalophus californianus*), ringed seal (*Phoca hispida*), bearded seal (*Erignathus barbatus*), spotted seal (*Phoca largha*), Atlantic bottlenose dolphin (*Tursiops truncatus*), and walrus (*Odobenus rosmarus*) (Dubey et al., 2003). *Toxoplasma gondii* infection or antibodies have also been found in several other southern marine mammal species (Fayer et al., 2004). However, in a study of more than 600 pinnipeds and cetaceans from the northeastern Atlantic (harp seal [*Phoca groenlandica*], ringed seal, hooded seal, and minke whale [*Balaenoptera acutorostrata*]), antibodies to *T. gondii* were not found (Oksanen et al., 1998).

Human toxoplasmosis in northern Quebec was statistically associated with consumption of raw caribou (*Rangifer tarandus*) meat, dried seal meat, and seal liver (McDonald et al., 1990). In Canadian barren ground caribou from the Northwest Territories and Nunavut, *T. gondii* antibody seroprevalences were 37% (n = 117) in animals of the mainland and 4% (n = 23) in animals from virtually uninhabited islands (Kutz et al., 2001). In Siberia seroprevalences of up to 21% were reported in reindeer (Beyer and Shevkunova, 1986). The overall seroprevalence of *T. gondii* in semidomesticated reindeer of Fennoscandia was 0.9%. A positive correlation with the degree of domestication of the reindeer was demonstrated, suggesting an association with domestic cats; seroprevalences of up to 25% were demonstrated in adult reindeer corralled for several months during winters (Oksanen et al., 1997). Furthermore, toxoplasmosis was diagnosed in 3 wild Arctic foxes (*Vulpes* (=*Alopex*) *lagopus*) found dead in Svalbard in 2000 (Sørensen et al., 2005) and examination of mammal and bird species, particularly Arctic foxes, demonstrated widespread *T. gondii* infection in the Svalbard ecosystem (Prestrud et al., 2007, 2008).

The polar bear (*Ursus maritimus*), with its circumpolar Arctic distribution (Amstrup, 2003), is a vulnerable species (Schliebe et al., 2006), potentially threatened by factors such as chemical pollution (Skaare et al., 2000; Lie et al., 2004; Muir et al., 2006) and global warming (Macdonald et al., 2005). In many regions, including Svalbard, the polar bear has been protected for several decades. The polar bear is at the top of the Arctic marine food web, with ringed seals and bearded seals as the main prey (Stirling and Archibald, 1977; Smith, 1980; Derocher et al., 2002). A diversity of other food items, such as harp seals, walruses, belugas (*Delphinapterus leucas*), narwhals (*Monodon monoceros*), reindeer, geese and other birds, fish, and people's food supplies and garbage, are also on the menu (Derocher et al., 2000, 2002; Amstrup, 2003).

Antibodies against *T. gondii* were demonstrated in polar bears from the Beaufort and Chukchi seas and the Russian Arctic between 1982 and 1999 (Rah et al., 2005). The overall se-

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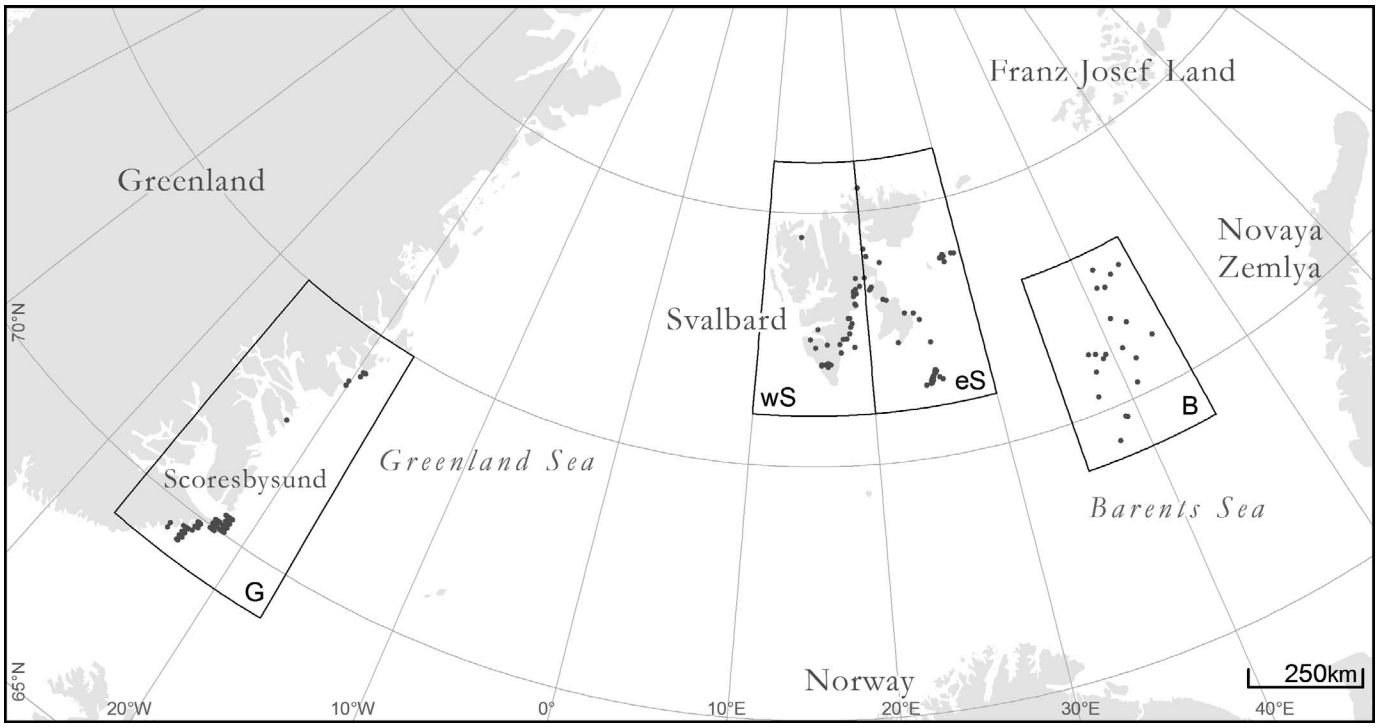

FIGURE 1. Sampling area of polar bears (*U. maritimus*) in Svalbard (with subregions wS: Svalbard west; eS: Svalbard east; and B: Barents Sea) and East Greenland tested for antibodies against *T. gondii*.

roprevalence in these 500 animals was 6%, with annual and regional differences.

The present study was aimed at determining antibody levels against *T. gondii* in polar bears from the Svalbard region including areas of the Barents Sea and from East Greenland, and identifying factors associated with the presence of antibodies.

MATERIALS AND METHODS

Animals and blood samples

The Svalbard polar bear population is monitored by Norwegian authorities, and annual expeditions by the Norwegian Polar Institute involve chemical immobilization with subsequent release of animals, for purposes such as biometry measuring, satellite tracking, and blood sampling. Serum samples from such animals have been assayed for antibodies against *Brucella* spp. and various viruses (Tryland et al., 2001, 2005). Tissue and blood samples from the East Greenland polar bears used in the present study were collected originally for the purpose of studying possible effects of contaminants on health of polar bears in this area (Dietz et al. 2004; Sonne et al. 2004; Sonne, Dietz et al., 2005; Sonne, Riget et al., 2005). The present study encompassed 419 polar bears from Svalbard and the Barents Sea (35 subadults and adults from Svalbard west, 272 from Svalbard east, and 35 from the Barents Sea; collectively hereafter ''Svalbard'') and 108 polar bears from East Greenland (hereafter ''Greenland'') (Fig. 1).

The Svalbard bears were chemically immobilized from helicopter by remote injection of a drug-filled dart (Stirling et al., 1989) and subsequently released, during the period 1990–2000. Of the 419 bears, 22 were recaptured 1–6 (mean 2.1, SD 1.2) yr later (total of 441 captures and samplings). Of the 441 captures, most were from the last 3 sampling years (41.4% in 1998, 15.4% in 1999, and 19.0% in 2000), making analyses of temporal trends irrelevant. Of the 419 bears, 31 were mothers accompanied by 1 or 2 cubs (in total 46) from which samples also were taken when captured (all in 1998 and 1999). The age by the first capture ranged from about 3 mo (cubs of the year) to 28 yr (mean 9.5 yr, SD 6.7), and the proportion of females was 48.2%. For the Svalbard polar bears, the capture site latitude and longitude range was 74°–81°N, 15°–44°E, respectively, involving a distance of nearly 700 km between the most western and eastern capture sites.

To look for spatial patterns within the Svalbard group, we divided the samples into 3 geographic subgroups based on the following longitudes and latitudes: Svalbard west: 74°–81°N, 15°–20°E, Svalbard east: 74°–81°N, 20°–30°E, Barents: 74°–77°N, 35°–45°E.

The 108 Greenland bears were harvested by subsistence hunting during 1999–2001 (84%) and 2003–2004 (16%), with age ranging from cubs of the year to 28 yr (mean 6.8, SD 6.1). The proportion of females was 48.1%.

Blood from the Svalbard bears was drawn from a femoral vein into heparinized, evacuated blood-collecting tubes, and the samples were transported to a field laboratory where plasma was pipetted off after centrifugation (1,000 *g*). For the Greenland bears, blood was collected and left to clot in the collection tube, and serum was pipetted off. At below freezing temperatures, the blood was frozen in the tube, and hemolyzed serum was pipetted off later after thawing and centrifugation. Samples were stored frozen at −18 C for subsequent analysis.

Age determination

The age of more than 1-yr-old Svalbard bears was determined by counting the cementum growth layers in a removed premolar tooth (Calvert and Ramsay, 1998). For the Greenland bears, the age was determined by counting the annual growth layers in the cementum of the I_3 after decalcification, thin sectioning (14 μm), and staining with toluidin blue (Hensel and Sørensen, 1980; Dietz et al., 1991; Kirkegaard, 2003). Aging from teeth in polar bears is not exact; a slight inaccuracy in aging has been shown for Svalbard bears, and it was shown that inaccuracy partly could vary because of differences in interpretation between different laboratories (Christensen-Dalsgaard, 2006). It is thus possible that age estimates are not fully comparable between Svalbard and Greenland.

Assaying for antibodies against *T. gondii*

The Svalbard samples from 1990 to 1998 (n = 285) were assayed at a dilution of 1:25 using the *T. gondii* modified agglutination test (MAT) with dithiothreitol (DTT) (Oksanen et al., 1998). Because of the temporary unavailability of reagents, the Svalbard 1999 and 2000 (n =

134) samples and the Greenland samples were assayed using a commercial agglutination test kit, Toxo-Screen DA (bioMérieux, Marcy l'Etoile, France), at a dilution of 1:40, including treatment of sera with 2-mercaptoethanol, according to the manufacturer's instructions. Samples giving agglutination at dilution 1:25 or higher were interpreted as positive and were assayed further for end titer determination, by 2-fold serial dilutions of each sample starting with 1:25 and ending with 1:3,200 (Svalbard 1990–1998 samples), or starting with 1:40 and ending with 1:1,280 (Svalbard 1999 and 2000 and Greenland samples). Results are not shown. The antigen used (whole *T. gondii* tachyzoites) and the principle of the test (agglutination of tachyzoites by specific IgG antibody, after destruction of IgM by DTT or 2-mercaptoethanol) were essentially identical for the 2 assay protocols. To ensure the agreement and thus the applicability between results from the 2 MAT protocols, 92 randomly selected samples from 1990–1998 already tested with DTT were retested using the commercial test. The kappa statistic was determined to 0.94, demonstrating agreement between results sufficient to justify the interchange between the 2 MAT protocols.

Statistical analysis

To identify factors associated with infection, analyses of contingency tables with frequencies or logistic regression analysis with *T. gondii* seropositivity as the dependent factor and age, sex, year of capture, and area (Greenland or Svalbard, and subgroups within Svalbard) as independent factors were performed. Analyses were done using the statistical software program R (version 2.6.0, R Development Core Team 2007).

RESULTS

Prevalence in cubs

We first compared the antibody prevalence of dependent cubs (less than 2-yr-old) to that of older animals (subadults and adults). The number of seropositive samples among dependent cubs was small: Only 3 cubs of the year and 3, 1-to-2-yr-old cubs were sampled from Greenland; none of them was seropositive. In Svalbard only 1, a male from 53 cubs (24 females and 29 males) of the year, was seropositive. One of 14 female and 1 of 9 male yearlings were seropositive. Thus, in total, there were 3 positives of 83 dependent cubs (3.6%). Because of the low number of seropositive animals among dependent cubs, and the low sample size of cubs from Greenland making analyses between areas impossible for this age group, we decided to use only bears of 2 yr of age, or older, in further analyses.

Prevalence compared to age, sex, and population

Among all the independent animals (n = 444) of both populations (2 yr and older), 95 were seropositive (21.4%). Table I illustrates the distribution of sample sizes and prevalence of *T. gondii* regarding to sex and population within different age groups. The prevalence was higher in Svalbard (24.3 %, n = 342) than in Greenland (11.8 %, n = 102), $\chi^2 = 6.58$, 1 d.f., $P = 0.010$). A logistic regression analysis (logit[prevalence *T. gondii*] = age + sex + age × sex) revealed neither effect of sex nor effect of the interaction term (P − values > 0.30, backward selection). However, there was a marked effect of age, with an increasing prevalence in the older animals (Table I). The best model on the age effect involved a polynomial term: logit[prevalence *T. gondii*] = −3.131 [SE = 0.517] + 0.250 [SE = 0.084] × age − 0.006 [SE = 0.003] × age^2 (age: dev = 20.01, d.f. = 1, $P < 0.001$; age^2: dev = 4.45, d.f. = 1, $P = 0.035$).

No significant relationship between sex and prevalence was seen when including both populations. However, when looking at the populations separately, prevalence among males (28.7%, n = 178) was higher than for females (19.5%, n = 164) in Svalbard, whereas prevalence was higher among females (18.0%, n = 50) than among males (5.8%, n = 52) on Greenland. The females in both populations had very similar prevalences. The interaction term between sex and population was significant (logistic regression, dev = 6.52, 1 d.f., $P = 0.011$). Average ages of the animals were higher in Svalbard (females: mean 10.8 yr, SD 5.4, males: mean 12.3 yr, SD 5.7) as compared to Greenland (females: mean 7.5 yr, SD 6.2, males: mean 6.8 yr, SD 6.0). The interaction could not be explained by the age differences only; in a model with logit (prevalence *T. gondii*) = age + age^2 + sex + population + sex × population, the interaction term was still significant (dev = 5.01, $P = 0.025$, see also Table I). It is also worth noticing that despite a higher total prevalence among males than females, none of the 48 young males (2-to-5-yr-old) was seropositive compared to 8 of 58 among females (Table I), although this did not result in a positive age × sex interaction (see above).

TABLE I. Distribution of sample sizes (n), number with antibodies against *T. gondii* (Pos), and percentage of samples with antibodies (%Pos) divided on population, sex, and age classes (in yr).

		Females			Males		
Location	Age	n	Pos	%Pos	n	Pos	%Pos
Svalbard	<2	38	1	2.6	39	2	5.1
	2–5	28	4	14.3	19	0	0
	6–10	60	8	13.3	54	15	27.8
	11–15	49	14	28.6	54	16	29.6
	>15	27	6	22.2	51	20	39.2
Greenland	<2	2	0	0	4	0	0
	2–5	30	4	13.3	29	0	0
	6–10	8	2	25	16	2	12.5
	11–15	4	1	25	2	0	0
	>15	8	2	25	5	1	20

Comparing *T. gondii* prevalence in cubs and their mothers

Of the 46 cubs for which samples also were available from the respective mothers (all from Svalbard), 1 was seropositive (titer 1:50) (mother: 21-yr-old and seronegative). Of the 31 mothers captured with cubs (age range of mothers: 7-to-21-yr-old), 7 (22.6%) (age range 9-to-19-yr-old) were seropositive (end titers 1:25 for 1 and higher than 1:1,280 for another, the others in between). The cubs of all the 7 seropositive mothers, 12 cubs in total (5 had 2 cubs each, 2 had 1 cub each) were seronegative. All the 46 cubs were captured in the second half of April or in early May.

Toxoplasma gondii prevalence in bears captured more than once

Of the 22 recaptured bears (all from Svalbard, age range 1-to-22-yr-old, mean 11.4, SD = 5.7), 4 (18.2%) were seropositive by the first capture. By the second capture, 1–6 yr later, the status (seropositive or negative) of all 22 was unchanged. One of the 4 seropositives (male, 8-yr-old by first capture) had titers of 1:25 at the first capture (1995) and 1:50 when recap-

tured (1998). The second (female, 1-yr-old), had a titer of 1:50 at both captures (1995, 1996). The third (male, 10-yr-old) had titers of 1:100 at the first capture (1996) and 1:800 when recaptured (1998). The fourth (female, 21-yr-old) had titers of 1:50 at the first capture (1998) and 1:640 at recapture (1999).

Geographic distribution of *T. gondii* among the Svalbard samples

Prevalence of antibodies against *T. gondii* in the Svalbard population among subadults and adults varied from 11.4% (n = 35) in the Barents Sea to 25.4% (n=272) and 28.6% (n = 35) for Svalbard east and Svalbard west, respectively. The differences were not significant (χ^2= 3.67, 2 d.f., P = 0.160).

DISCUSSION

Overall prevalence of antibodies

Based on the scarce earlier knowledge of *T. gondii* in mammals of the northeastern Atlantic, the proportion of seropositives as high as demonstrated here (particularly for Svalbard) was somewhat surprising. The kinetics of antibodies to *T. gondii* in polar bears is not known, but seropositivity indicates previous contact, or ongoing infection, with the parasite. The MAT is generally regarded as very specific for *T. gondii*, with few or no cross-reactions with antibodies against other tissue cyst-forming coccidia, such as *Neospora caninum*, the closest relative to *T. gondii* hitherto known (Dubey, 1997; Hilali et al., 1998). In 500 polar bears captured in the Beaufort and Chukchi seas and the Russian Arctic, 1982–1999, the overall prevalence of antibodies against *T. gondii* was 6% (Rah et al., 2005). However, in Russian adult female polar bears captured from the Kara Sea to the Barents Sea and Franz Josef Land area, the prevalence was 23% (7 of 30), which is comparable to our findings. In that study the annual prevalence in the entire material varied between 0 and 25%. During the first 9 yr, 1982–1990, seropositive animals were detected during 4 of the yr, and of the last 9 yr, 1991–1999, seropositives were seen in 8 of the years; however, a statistically significant increase was not seen. Highest seroprevalence was seen in cubs, i.e., 2 positives of 23 tested (8.7 %). In an earlier study in East Greenland in an area about 250 km north of Jamesonland (71N, 23W), no seropositive animal was found among 21 polar bears captured (Clausen and Hjort, 1986). Because of the small sample size, it is not possible to conclude if this infers an increase in prevalence in East Greenland. Even less is known about previous *T. gondii* prevalence in Svalbard polar bears.

Age and sex differences

The proportion of *T. gondii* seropositive polar bears increased significantly by age for both the Svalbard and Greenland subpopulations, which was in concordance with findings from the Alaskan and Russian Arctic (Rah et al., 2005). The finding that only 3 of the 83 (3.6%) dependent bears (age less than 2-yr-old) were seropositive, combined with the finding of a high prevalence in other age groups (21.4%), indicated that transfer from mother to offspring is not important as a route of transmission of *T. gondii* in polar bears. This was even more strongly supported by the finding that all the 12 cubs of the 7 seropositive mothers were seronegative, demonstrating the absence of maternal antibodies by the time they were captured in April. Maternal antibodies could have been present in the cubs earlier, after placental or colostral transfer, and subsequently been diminished to levels below detection limits. The seropositive cub and its mother were captured in mid-April, and the seronegativity of the mother indicates that the infection in the cub probably originated from food intake.

The male gender-related difference in seroprevalence between the bears from Svalbard and Greenland seems likely induced by sex- and area-specific feeding behavior. It is well known that males and females within areas can have different prey species preferences (Ramsay and Stirling, 1986; Stirling and Derocher, 1990). A study of fatty acids in polar bears and different prey species indicated that adult male polar bears in some areas of Canada preyed much more on bearded seals and walruses than females and younger polar bears (Thiemann et al., 2007). Prevalences of *T. gondii* in bearded seals and walruses are not known for eastern Greenland, but no seropositive was found among a number of both harp seals and hooded seals (Oksanen et al., 1998). Little information is available on the extent to which species other than ringed seals are being preyed upon by the East Greenland polar bears. If males in the area are more likely to mainly feed on seals with low prevalences of *T. gondii*, and females also to a large degree prey on more terrestrial prey species with perhaps higher prevalences of *T. gondii*, it could possibly explain the difference. In Svalbard the high prevalence found in Arctic foxes could be caused from feeding on birds (Prestrud et al., 2007), indicating that a terrestrial source may indeed be likely. It is still not easy to explain why male polar bears in Svalbard have very high prevalence of *T. gondii*. It could be that *T. gondii* is more frequent in some marine mammal prey species there as compared to Greenland, e.g., bearded seals and walruses. One walrus was positive of 17 in Svalbard (Prestrud et al., 2007). Other possible explanations are that males in Greenland and Svalbard hunt different prey, or that intraspecific predation or scavenging is more common in Svalbard, where no hunting regulates the population. No significant gender-related difference in prevalence was found among polar bears from the Beaufort Sea and Russian Arctic (Rah et al., 2005).

Source of infection

In Svalbard cats are banned by the Norwegian authorities; however, a few cats may exist in Russian mining communities. Thus, the possibility of cats as a source of infection for polar bears cannot totally be excluded. Nonetheless, the existing cat population is very limited and local, and the proportion of seropositive polar bears is rather high, indicating that polar bears are commonly infected with *T. gondii*. It would, therefore, be inconceivable to assume that the few cats would play a major role in the epidemiology of *T. gondii* in the vast high Arctic. This is apparently the case in East Greenland as well. In populations of other species of bears living closer to human settlements and, therefore, with closer contact to cats, most often greater, sometimes a similar, but occasionally even lower, percentage of seropositivity has been reported (Briscoe et al., 1993; Chomel et al., 1995; Dubey et al., 1995; Zarnke et al., 1997; Dunbar et al., 1998; Nutter et al., 1998; Zarnke et al., 2000).

It has been proposed that there might exist a yet unknown

life cycle for *T. gondii* in the Arctic, independent of cats (Measures et al., 2004). However, recent genetic studies of *T. gondii* isolated from a Svalbard Arctic fox in a population where the prevalence of antibodies has been shown to be high, 43% (Prestrud et al., 2007), indicated no difference of the parasite compared to the strains most common in Europe, which rather supports an hypothesis of parasite inflow from continental Europe (Prestrud et al, 2008).

An inflow of *T. gondii* from continental Europe might take place by barnacle geese, *Branta leucopsis*, or other migratory birds. Further spread of the parasite might occur through scavenging and cannibalism as previously suggested for the Svalbard Arctic fox (Prestrud et al., 2007, 2008). Cannibalism is considered the main route of infection for *Trichinella* spp. in polar bears (Forbes, 2000), and it is plausible that similar transmission may take place for *T. gondii*. However, absence of hard evidence on marine sources of *T. gondii* is not equal to evidence of absence, and further studies are needed to reveal transmission patterns of *T. gondii* in the marine ecosystem.

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